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# Melanism in the grass snake *Natrix natrix* (Linnaeus, 1758) from the Danube Delta Biosphere Reserve, Romania

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## Abstract

Animal colouration has a significant ecological role in defence, reproduction, and thermoregulation. In the case of melanism, it is a complex topic. Besides potential disadvantages such as higher risk of predation, melanistic ectotherms may have certain physiological advantages such as more efficient thermoregulation in colder climates and thus, reduced basking time. The common grass snake (*Natrix natrix*) is a widespread species throughout Europe and Asia. It exhibits a wide range of colour polymorphisms, from olive to dark grey, even albinistic and melanistic. Between 2016 and 2021, we conducted fieldwork in the Danube Delta Biosphere Reserve (DDBR) with the aim to document the geographic range of melanistic grass snakes. We categorised the melanistic expressions of *N. natrix* individuals as melanotic, completely melanistic, and partially melanistic. Melanistic snakes were encountered in all six localities visited, suggesting that the occurrence of melanistic grass snakes in the DDBR is geographically widespread. We observed both juveniles (n=2) and adults (n=11) with melanism, suggesting that individuals are born melanistic. However, the proportion of melanistic individuals in the general population of *N. natrix* from the DDBR is unknown. Only at Histria locality we studied the proportion of melanism in the grass snake population and 6.3% of the snakes caught were melanistic. Body size comparisons are not statistically significant because of the low sample size. The *N. natrix* melanistic morph's geographical distribution in the DDBR is most likely due to an interaction of climate and habitats, which offer a thermal advantage in the face of predation pressure.

## Key Words

colour morphs, ectotherms, melanotic, polymorphism

## Introduction

Animal colouration provides ecological roles to organisms such as the capacity to warn predators, increase crypsis, signal potential mating partners, and is subject to strong selective pressures (Clusella-Trullas et al. 2007). In ectothermic vertebrates the intra- and interspecific variation in colour provides thermoregulatory opportunities to exploit niches that maximise their thermal performance optimums (Clusella-Trullas et al. 2007; Geen and Johnston 2014). For instance, darker colour is supposedly favoured in cooler climates since

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dark individuals (i.e., lower skin reflectance) have an advantage under low temperature conditions, as they heat up faster than lighter individuals (Clusella-Trullas et al. 2007; Hodges 2018). Melanism occurs when the quantity of skin melanin pigment is found in large amounts at the expense of other colours (Majerus 1998; van Grouw 2017; Mărănducă et al. 2019). This can lead to animal populations with complete melanistic individuals that display an entirely black colour phenotype or variants such as melanotic (nearly entirely expressed melanism) or pseudo-/partial- melanism (Majerus 1998; Zuffi 2008; Domeneghetti et al. 2016). Various melanin concentrations in animal populations led to varying degrees of darker pigmentation leading to continuous intraspecific polymorphism (True 2003). The drivers of melanistic polymorphism are still subject to debate and research (Majerus 1998; Zuffi 2008; Domeneghetti et al. 2016). Laboratory experiments and field studies on ectotherms showed that melanistic morphs use different thermoregulation strategies to morphs with less melanin, such as different use of habitat and lower basking time. Sampling of *Vipera aspis* in the Swiss Alps had indicated that melanistic individuals prefer cooler microhabitats with higher vegetation cover, compared to non-melanistic ones (Muri et al. 2015). Thus, melanistic morphs preserve their body temperature better in colder weather and through physiological and behavioural adaptations are protected against overheating in warm weather conditions (Gibson and Falls 1979; Clusella-Trullas et al. 2007; Tanaka 2007; Muri et al. 2015). Studies on melanistic snakes showed that they are generally smaller than non-melanistic individuals from the same population and prone to increased predation, which could indicate that melanistic individuals could potentially have minimal thermal benefits compared to non-melanistic individuals (Madsen 1987; Bury et al. 2020).

Melanism is generally not rare in certain snakes, especially in colder environments (Clusella-Trullas et al. 2007; Lorioux et al. 2008; Zuffi 2008). Across Europe there have been recorded melanistic individuals in different snake species such as *Zamenis longissimus* (Zadravec and Lauš 2011), *Coronella austriaca* (Nash et al. 2016), *Vipera berus* (Terhivuo 1990; Strugariu et al. 2009; Nash et al. 2016). Melanistic individuals have also been recorded in the semi-aquatic *Natrix* species (Baran 1976; Naumov 2007; Ajtić et al. 2013; Gvozdenović and Schweiger 2014; Jablonski and Kautman 2017).

The grass snake *N. natrix* is a species known for its polymorphism, with a wide range of colours and morphs which have been reported within its range. Dorsal pattern can range from typical olive-green, brown or greyish to individuals with rows of black spots or bars, or double yellow stripes (Fuhn and Vancea 1961; Thorpe 1979; Kabisch 1999; Speybroeck et al. 2016).

We still have a limited understanding of the distribution and frequency of the melanistic morph in *N. natrix* populations throughout its range. Reports of melanistic individuals of grass snakes have been made from Turkey (Baran 1976; Habiboğlu et al. 2016; Yenmiş et al. 2022), Bulgaria (Naumov and Tomović 2005; Stojanov et al. 2011; Mollov 2012), Serbia (Bjelica and Anđelković 2021), Croatia (Zadravec and Lauš 2011), Montenegro (Gvozdenović and Schweiger 2014), Bosnia and Herzegovina (Bašić and Zimić 2016), Slovakia (Jandzik 2004), Poland (Błażuk 2007; Kolenda et al. 2017; Bury et al. 2020). In Romania, there are only three general literature mentions of melanistic *N. natrix*, all of them from the Danube Delta Biosphere Reserve (DDBR) (Fuhn and Vancea 1961; Halpern et al. 2002; Zamfirescu et al. 2010).

The aim of our paper is to document the geographic range of melanistic individuals of grass snakes (*N. natrix*) from the DDBR. We also discuss possible advantages and disadvantages for the occurrence of melanism in the study region.

# Methods

## Study area

The Danube Delta is composed of a network of channels, lagoons, marshes, levees, and three main Danube river lobes (Chilia to the north, Sulina in the middle and Sfântul Gheorghe to the south) and the Razelm-Sinoe lagoon complex (Vespremeanu-Stroe et al. 2017). The DDBR has an average altitude of 0.52 m above mean Black Sea level, with the highest point at 12.4 m altitude, and covers an area of 5800 km$^2$ and supports rich biodiversity, with numerous populations of bird and reptile species (Gâştescu 2009; Zamfirescu et al. 2010; Marinov et al. 2019). According to the Köppen-Geiger classification system, the region is classified as a C climate, warm temperate, fully humid with hot summers (Kottek et al. 2006). The DDBR is influenced by the Black Sea and is characterised by torrid summers, very cold winters and is considered the windiest and sunniest region of Romania (Ciulache and Torică 2003; Gâştescu 2009). The habitats are diverse, ranging from anthropic settlements, agricultural fields and pastures to sand dunes with reeds and a few areas with forests, in close proximity to water bodies (Doroftei 2013). 12 reptile species have been recorded here (*Emys orbicularis*, *Testudo graeca*, *Eremias arguta*, *Lacerta agilis*, *Lacerta viridis*, *Podarcis tauricus*, *Coronella austriaca*, *Elaphe sauromates*, *Dolichophis caspius*, *Natrix natrix*, *Natrix tessellata*, *Vipera ursinii*); of these, *N. natrix* is the most common species due to the suitable habitats (Zamfirescu et al. 2010; Cogălniceanu et al. 2013).

## Data collection and analysis

We conducted opportunistic and transect surveys of melanistic individuals in the areas accessible by foot, between 8:00 – 14:00h, during the snake's active season

(March-October), from 2016 to 2021 throughout the DDBR. Melanistic grass snakes were visually searched for and caught by hand when possible. Upon capture, we measured snake's body mass (BM) using a spring / digital scale (0.1 g accuracy) and photographed alongside a graph paper, using DSLR cameras with standard lenses (Nikon D7100 and Olympus OM-D E-M1 MarkII). The snakes' snout-vent length (SVL) was then measured in ImageJ (Rasband 1997). The location of each melanistic individual was recorded using Orux Maps and Geo Tracker applications for mobile phone, using geographic coordinates in datum WGS84. At one 32 ha locality, the Histria Archaeological Complex (Fig. 1), the methodology described above was applied to both melanistic and non-melanistic grass snakes.

Depending on the morphological expression of melanistic characteristics, we categorised the melanistic morphs of grass snakes following Zuffi (2008) as:

- completely melanistic: complete melanism expressed, with a few ventral white scales still retained (Fig. 3C);
- melanotic: where melanism is nearly entirely expressed, with a few morphological characteristics which break the melanistic expression, in grass snakes – a few dorsal scales coloured with dark brown pigment (Figs 2, 3B);
- partially melanistic: where the normal colour pattern has an increased black pigmentation (Fig. 3A).

For the statistical analysis between groups of melanistic and non-melanistic grass snakes from Histria locality we have applied a One-Way Anova test.

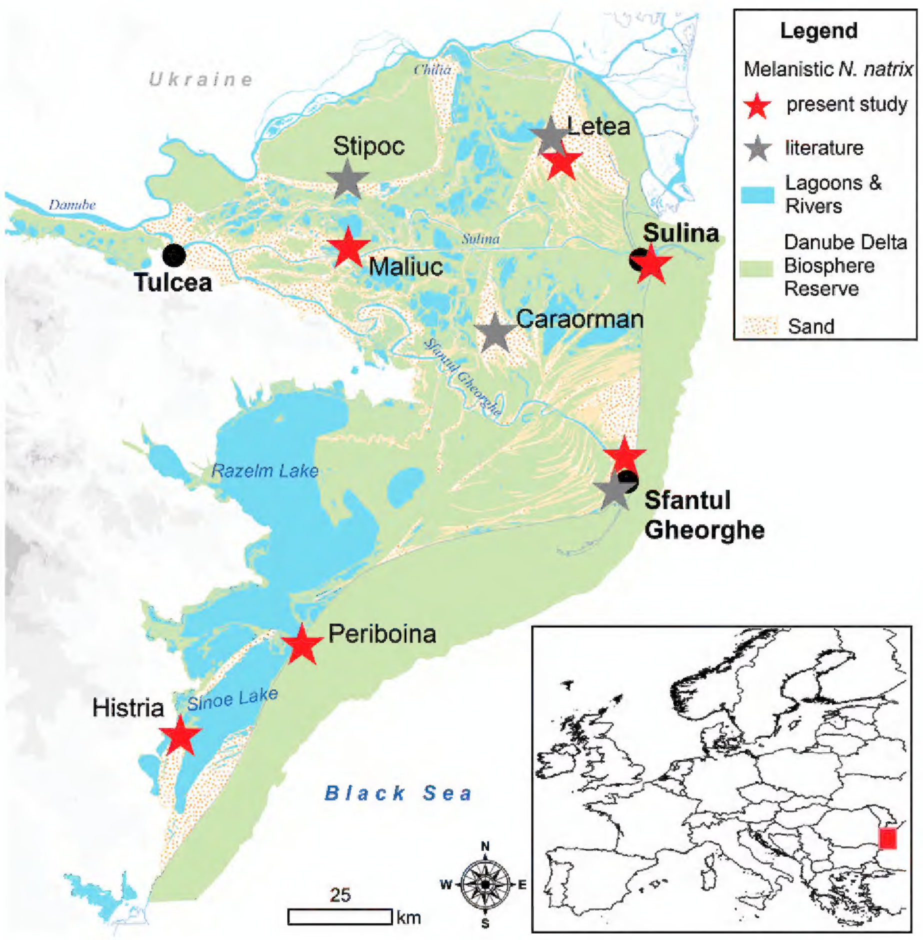


**Figure 1.** Danube Delta Biosphere Reserve and the locations of the melanistic grass snakes *Natrix natrix* found during our study and in literature (map source: OpenStreetMap.org and geo-spatial.org).

## Results

We found 13 melanistic individuals in close proximity to six localities: Letea, Maliuc, Sulina, Sfântu Gheorghe, Periboina, and Histria (Fig. 1). All melanistic snakes were found in habitats characterised by sand dunes with reed vegetation, surrounded by water bodies such as lakes, canals or the coastal area. Four were adult females (SVL between 580 – 870 mm), six were adult males (SVL between 500 – 882 mm) and two were juveniles (SVL 185–198 mm) (Table 1). One adult male from Periboina locality lacks morphometrics data because it was found opportunistically by colleagues. An adult found in Sulina locality, with unknown sex was found dead on the road in a state that precluded measuring.

**Table 1.** Characteristics of the melanistic individuals of grass snakes *Natrix natrix* found in DDBR between 2016 and 2021. Abbreviations as follows: Localities L-Letea, M-Maliuc, S-Sulina, SG-Sfantu Gheorghe, H-Histria, P-Periboina (Fig. 1); Melanism CM-completely melanistic, PM-partially melanistic, Ml-melanotic; Age A-Adult, J-Juvenile; *Dead on road.

| Locality | Date | Sex | Age | SVL (mm) | Weight (g) | Coordinates | Melanism | Figure |
|---|---|---|---|---|---|---|---|---|
| L | 14.05.16 | M | A | 500 | 42.9 | 45°17.16'N, 29°31.44'E | CM | |
| L | 04.06.17 | – | J | 198 | 30 | 45°17.16'N, 29°31.44'E | CM | |
| M | 16.08.17 | F | J | 185 | 18.8 | 45°12.00'N, 29°6.66'E | PM | |
| S | 06.06.17 | – | A* | – | – | 45°9.36'N, 29°39.24'E | CM | |
| SG | 17.06.17 | F | A | 870 | 284.8 | 44°53.76'N, 29°35.58'E | CM | |
| SG | 22.07.17 | M | A | 882 | 191.4 | 44°53.76'N, 29°35.58'E | CM | |
| SG | 22.07.17 | M | A | 590 | 95.9 | 44°53.76'N, 29°35.58'E | Ml | |
| SG | 22.07.17 | M | A | 641 | 81.6 | 44°53.76'N, 29°35.58'E | PM | 3A |
| P | 24.04.21 | M | A | – | – | 44°36.78'N, 28°55.80'E | CM | 3C |
| P | 01.05.21 | F | A | 680 | – | 44°36.84'N, 28°55.86'E | CM | |
| H | 13.10.18 | F | A | 625 | 76 | 44°32.82'N, 28°46.38'E | Ml | |
| H | 17.10.20 | M | A | 630 | 82 | 44°32.82'N, 28°46.50'E | Ml | 2 |
| H | 19.10.21 | F | A | 580 | 70 | 44°32.94'N, 28°45.72'E | Ml | 3B |

We encountered all three categories of melanism expression: 53.8% completely melanistic individuals (n=7), 15.4% partially melanistic (n=2), and 30.8% melanotic (n=4) (Table 1, Figs 2, 3).

At Histria we caught a total of 48 adult grass snakes, of which only three snakes were melanistic (6.3%). The three melanistic grass snakes from Histria had an average SVL of 611.66 mm and BM of 76.00 g. The non-melanistic grass snakes had an average SVL of 609.71 mm, and BM of 76.26 g (Table 2). The difference in body size between the two categories is statistically non-significant (df=1, F=0.00, p>0.05) for both SVL and BM.

**Figure 2.** Polymorphism observed in *Natrix natrix* populations from DDBR. Top common morph; middle double yellow stripes pattern; bottom melanotic morph (photo credit: E. A. Telea & G. Fănaru).

## Discussion

Melanistic grass snakes have been found in all six localities surveyed, four of these not mentioned before in literature (Fig. 1). Although *N. natrix* is a widespread species in Romania (Cogălniceanu et al. 2013), previous recorded melanistic were all observed in the DDBR, in four localities (Fuhn and Vancea 1961; Halpern et al. 2002; Zamfirescu et al. 2010). Literature data and our results suggest that melanism in *N. natrix* is geographically widespread in the DDBR.

We have encountered all three melanistic morph categories, as defined after Zuffi (2008), and as much as we could identify them, they have been previously reported in *N. natrix* across its range (Fuhn and Vancea 1961; Naumov and Tomović 2005; Zamfirescu et al. 2010; Zadravec and Lauš 2011; Mollov 2012; Gvozdenović and Schweiger 2014; Habiboğlu et al. 2016; Kolenda et al. 2017; Bjelica and Anđelković 2021; Yenmiş et al. 2022). Both the present study and recorded cases in literature of melanistic grass snakes rely on visual encounter surveys. Fuhn and Vancea (1961) mentioned that the most common melanistic morph of grass snakes in the Romanian DDBR was the melanotic phenotype. However, in our study, the completely melanistic one was more abundant and widespread, found in the majority of localities visited (Figs 1, 3C, Table 1). In literature, this morph seems to be the most commonly reported melanistic phenotype in grass snakes and has been reported before in Poland (Kolenda et al. 2017), Bulgaria (Naumov and Tomović 2005; Mollov 2012), Turkey (Habiboğlu et al. 2016), Serbia (Bjelica and Anđelković 2021), and in the Romanian DDBR (Zamfirescu et al. 2010). The melanotic phenotype, on the other hand, was the second most often encountered in our study, numerically and geographically (Figs 1, 3B, Table 1). This morph was also recorded in Turkey in a record published by Yenmiş et al. (2022). The third morph, the partially melanistic morph, was the least encountered numerically and in only two localities visited (Figs 1, 3A, Table 1). The partially melanistic grass snakes were previously recorded in grass snakes in Croatia and Montenegro (Zadravec and Lauš 2011; Gvozdenović and Schweiger 2014).

In the small sample size from Histria locality (Fig. 1, Table 2), melanistic and non-melanistic grass snakes did not differ in body length or body size. In a previous study, Bury et al. (2020) showed that the melanistic grass snakes are smaller than the typically coloured individuals of both sexes. The small size of melanistic snakes is counterintuitive, seeing as, generally, a thermoregulatory advantage leads to higher growth rates, larger body size, and greater reproductive success (Bittner and King 2003).

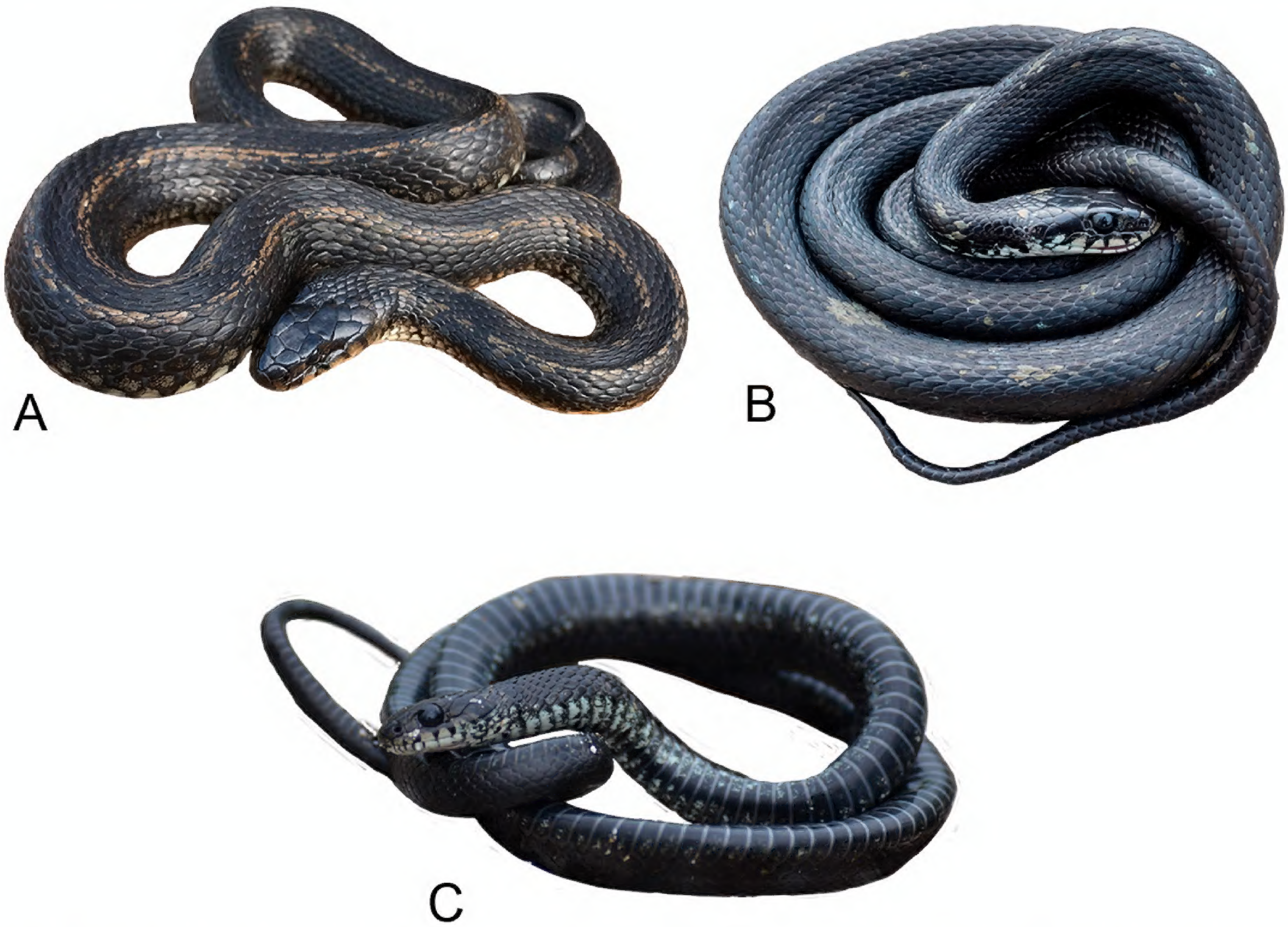

Figure 3. Examples of encountered melanistic morphs of *Natrix natrix*. **A.** dorsal view of partially melanistic morph with double yellow stripes pattern, Sfântu Gheorghe (photo credit: I. Gherghel); **B.** dorsal view of melanotic morph, Histria (photo credit: E.A. Telea); **C.** partial lateral and ventral views of completely melanistic morph, Periboina (photo credit: F. Stănescu).

Forsman (1995) identified no consistent difference in the proportion of time spent basking and maintaining higher temperatures between melanistic and zigzag individuals of *V. berus*. Such a situation could explain our population case: low altitude combined with the DDBR's climate and high-density vegetation habitats could offer some advantages for thermoregulatory behaviour for melanistic grass snakes against predation pressure, but not enough to make a considerable difference in higher growth rate opportunities between melanistics and non-melanistics. But because of the small sample size of grass snakes from this locality, we stress that further studies are needed for a better sample size in order to test differences in size and growth rate between the colour morphs.

**Table 2.** Body sizes of grass snakes *Natrix natrix* from Histria locality.

| | SVL (mm) min–max mean±s.e. | BM (g) min–max mean±s.e. |
|---|---|---|
| Melanistic (n = 3) | 580–630 | 70–82 |
| | 611.66±15.89 | 76.00±3.46 |
| Non-melanistic (n = 45) | 445–785 | 25–169 |
| | 609.71±11.26 | 76.26±4.73 |

All surveys were done during the day, from morning to early afternoon. All melanistic grass snakes we found were either hidden in reed vegetation or in close proximity to an abundant reed vegetation habitat. This habitat choice might have affected the success of our detection and capture of melanistic grass snakes. However, such behaviour and habitat choice might not have been accidental. Hypothetically, it could have been advantageous to the melanistic grass snakes as they could maintain a thermal benefit in microhabitats with less exposure to the sun (e.g., high-density vegetation), while also avoiding predator (and researcher) detection. From an evolutionary point of view, in snakes, melanism is established when the thermoregulatory advantage outweighs the risks (Clusella-Trullas et al. 2007; Broennimann et al. 2014). Studies have shown that at a population level melanistic snakes are more predated upon; because of their distinctiveness, predators have an easier task of singling out the dark individual (Andrén and Nilson 1981; Madsen 1987). At continental level, darker individuals of animals (i.e., individuals with higher concentration of melanin) tend to live in warm and humid climates (Gloger, 1833 in Millien et al. 2006). However, melanism is thought to be more advantageous in colder environments such as high-altitude habitats,

or even peninsular and coastal habitats characterized by cooler and more humid weather (Millien et al. 2006; Clusella-Trullas et al. 2007; Geen and Johnston 2014). In the case of the DDBR, the region is characterized by a warm, sunny and humid climate, with strong winds from the coastal regions. The DDBR is also known for a very high abundance and diversity of bird species. Although it is a suitable region for many birds, including species which prey upon snakes, such as *Circaetus gallicus*, *Circus* sp. and *Ciconia* sp. (Prejbeanu and Rada 2007; Gâştescu 2009; Marinov et al. 2019), the melanistic grass snake individuals have plenty of dense habitats suitable for hiding from potential predators. The abundant reed vegetation offers protection both against predators and overheating in the DDBR's warm and humid climate, but also protection against the characteristic cool, strong winds for this coastal region (Ciulache and Torică 2003; Kottek et al. 2006). The presence and abundance of *N. natrix* melanistic morph's geographical distribution in Romania in the DDBR is likely due to the interaction of climate and habitat, which offers a thermal advantage in the face of predation pressure.

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